transmitting HCMV from donor to recipient, allowing the use of standard cell separation techniques to enrich or deplete the stem cell product of specific cell types before transplantation into NSG hosts. Of particular interest are myeloid lineage cells, such as $CD34^+$ progenitor cells and $CD14^+$ monocytes, which have been previously described as harboring latent HCMV [11]. Defining the cell populations that harbor latent HCMV may lead to novel strategies to prevent transmission during PBSCT.

Why only 15%-20% of seropositive donors successfully transmit HCMV to seronegative recipients is not clear. The previous lack of an experimental model limited the analysis of transmission to retrospective clinical studies. The NSG mouse model now permits the experimental evaluation of factors inherent to the allograft that have been found to correlate with transmission in such studies [5]. In addition, this model provides a tool for testing novel hypotheses, such as whether the risk of transmission is related to the quantitative viral load in the allograft. Thus, the NSG mouse model provides a unique opportunity to gain further insight into the fundamental mechanisms of HCMV transmission and latency after PBSCT.

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Improved Survival with Ursodeoxycholic Acid Prophylaxis in Allogeneic Stem Cell Transplantation: Long-Term Follow-Up of a Randomized Study

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We report the long-term results of a prospective randomized study on the use of ursodeoxycholic acid (UDCA) for prevention of hepatic complications after allogeneic stem cell transplantation. Two hundred forty-two patients, 232 with malignant disease, were randomized to receive (n = 123) or not to receive (n = 119) UDCA from the beginning of the conditioning until 90 days post-transplantation. The results were reported after 1-year follow-up. UDCA administration reduced significantly the proportion of patients developing high serum bilirubin levels as well as the incidence of severe acute graft-versus-host disease (GVHD), liver GVHD, and intestinal GVHD. In the UDCA prophylaxis group, nonrelapse mortality (NRM) was lower and overall survival better than in the control group. After a 10-year follow-up, the difference in the survival and NRM in favor of the UDCA-treated group, seen at 1 year, was maintained (survival 48% versus 38%, $P = .037$; NRM 28% versus 41%, $P = .01$). A landmark analysis in patients surviving at 1 year post-transplantation showed no significant differences between the study groups in the long-term follow-up

in chronic GVHD, relapse rate, NRM, disease-free survival, or overall survival. These long-term results continue to support the useful role of UDCA in the prevention of transplant-related complications in allogeneic transplantation.

INTRODUCTION

Liver problems are common after allogeneic stem cell transplantation. Veno-occlusive disease of the liver (or sinusoidal obstruction syndrome) and liver graft-versus-host disease (GVHD) are the most serious complications. In 1996 to 1998, the Nordic Bone Marrow Transplantation Group carried out a prospective randomized study to evaluate the possible role of ursodeoxycholic acid (UDCA) in the prevention of liver complications [1]. UDCA is a hydrophilic bile acid constituting less than 5% of bile acids in the normal bile [2]. The proportion can be increased to 40% to 50% by oral administration [2]. The concentration of hydrophobic bile acids is thereby reduced [3-5]. Hydrophobic bile acids are toxic to liver parenchymal cells in direct contact that takes place in disorders damaging bile ducts, whereas hydrophilic bile acids are nontoxic. UDCA has also been shown to affect the release and expression of inflammatory cytokines [6,7] and the hepatocyte anion transporters of conjugated bilirubin [8], to activate the glucocorticoid receptor [9], to have immunomodulatory effects [2,7], and to stabilize hepatocyte membranes [10]. These effects may have a role in the prevention of liver problems.

In the present study, analyzed and reported after a 1-year follow-up [1], UDCA administration reduced significantly the proportion of patients developing high serum bilirubin levels as well as the incidence of severe acute GVHD, liver GVHD, and intestinal GVHD. The incidence of serious liver problems was lower than among the control patients. In the study group given UDCA prophylaxis, nonrelapse mortality (NRM) was significantly lower and overall survival significantly better than in the control group. The survival benefit was seen especially among the low-risk patients. No adverse effects of UDCA were observed.

The clinical benefits obtained with UDCA prophylaxis in the first year post-transplantation were marked, and a long-term follow-up was indicated to study whether the reduction in NRM and improvement in survival was maintained in the following years. The reduction in acute GVHD indicated a possible immunological effect that might have an influence on chronic GVHD, relapse rate, or the incidence of secondary neoplasms. Therefore, a long-term follow-up was carried out.

METHODS

The present study was a prospective, randomized, open-label multicenter study. All patients undergoing allogeneic hematopoietic stem cell transplantation during the period from January 1996 to November 1998 at the 3 participating centers, a total of 244 patients, were randomized (1:1) to receive or not to receive UDCA. The randomization was carried out in blocks of 4 in sealed opaque envelops to ensure balance between the groups. One patient in each arm did not undergo the transplantation, 1 because of death and 1 due to cancellation of the procedure. The randomization was stratified according to the disease category (low or high risk), type of donor (HLA-identical sibling, other related, or unrelated), conditioning (total body irradiation, yes or no), and center. Low-risk patients were those with acute leukemia in first remission, chronic myeloid leukemia in first chronic phase, aplastic anemia, or hereditary disorder. All others were high-risk patients. The primary endpoint was the maximum total bilirubin concentration during the first 90 days [1]. The patients gave their informed consent, and the study was approved by the ethics committee of each participating center. Patients were informed and recruited by the treating physician or responsible investigator.

The long-term outcome of 242 patients was studied. In addition, separate analyses were performed among the 156 patients who survived at 1 year post-transplantation to study the possible effect of UDCA prophylaxis on the later outcome.

One hundred twenty-three patients were randomized to receive and 119 not to receive UDCA (Leiras, Helsinki, Finland). The dose was 12 mg/kg/day, given from the day preceding the conditioning until day 90 after the transplantation. Two hundred thirty-two patients had a malignant disease. Seventy-two patients had acute myeloid leukemia, 68 chronic myeloid leukemia, 50 acute lymphatic leukemia, 23 myelodysplastic syndrome, 5 multiple myeloma, 4 chronic lymphatic leukemia, 4 non-Hodgkin lymphoma, 3 myelofibrosis, and 13 other disease. Seventy-five patients in the UDCA group and 65 patients in the control group were low-risk patients. Detailed demographics of the patients were described in the original article [1]. All patients with a malignant disease received standard myeloablative conditioning, in 219 cases based on total body irradiation. Six patients in the UDCA-treated group and 11 in the control group received busulfan. The donor was an HLA-identical sibling in 55%, unrelated in 45%, and other family member in 1% of the transplantations. The graft was bone marrow in 79% and peripheral blood stem cells in 21% of the cases. GVHD prophylaxis consisted of cyclosporine and a short course of methotrexate with or without corticosteroid in all but 4 patients.

Data were collected retrospectively after the initial 1-year prospective phase. The median follow-up of living patients was 155 months (range, 37 to 184 months). One patient in each study group was lost for follow-up, at 56 and 37 months. The participating centers record the status of their patients annually for 10 years after the transplantation and thereafter every second year. We report here the follow-up for 10 years. The parameters studied were the incidence and grade of chronic GVHD [11], significant liver problems, NRM, relapse rate, disease-free survival, overall survival, secondary malignancies, and causes of death.

The cumulative incidence of chronic GVHD, relapse, NRM, and survival were analyzed by Kaplan-Meier curves and compared by the log-rank test. Relapse and NRM were assessed using a competing risk analysis [12,13]. The proportions between the groups were compared by the chi-square test or Fisher's exact test. Because of multiple testing of clinical endpoints, *P* values adjusted according to Benjamini-Hochberg false discovery rate [14] are also given.

RESULTS

The survival difference seen at 1 year in favor of the UDCA-treated group (71% versus 55%) remained similar in the long-term follow-up. At 10 years, 48% of patients given UDCA and 38% of control patients were alive ($P = .037$, adjusted for multiple testing .047) (Figure 1A). The survival difference was marked among the low-risk patients, 63% versus 46%, $P = .019$ (adjusted .038) (Figure 1B), whereas there was no difference among the high-risk patients, 25% versus 29% (Figure 1C). The difference in NRM observed at 1 year (UDCA prophylaxis 19% versus control group 34%) was maintained through the follow-up. In the total patient material, the cumulative incidences of NRM at 10 years were 28% versus 41%, respectively ($P = .01$, adjusted .038) (Figure 2).

There was no significant difference in the incidence or severity of chronic GVHD; the cumulative incidence was 58% in the UDCA group and 68% in the control group ($P = .47$). In the long-term follow-up, past 1 year after the transplantation, there were no significant differences in new liver problems between the study groups.

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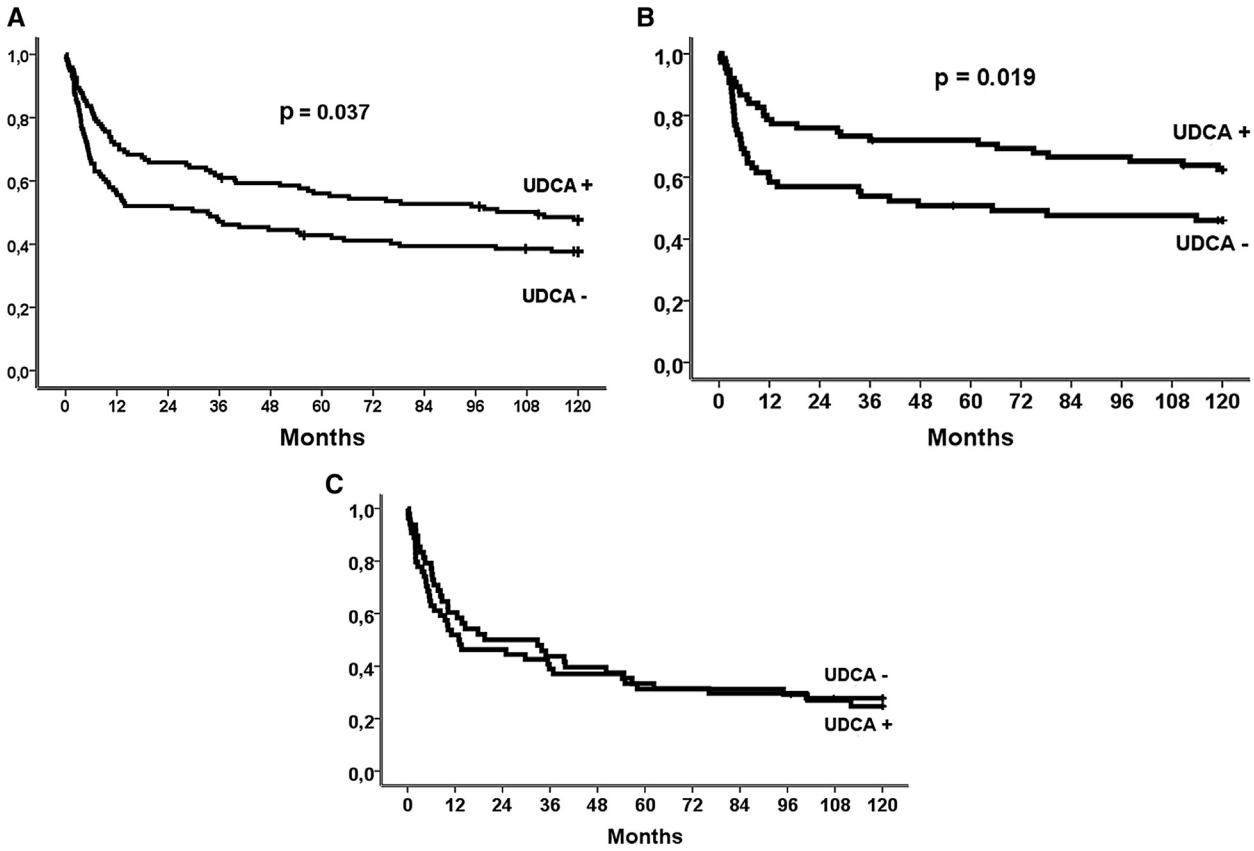

Figure 1. Overall survival in the whole patient group (A), in low-risk patients (B), and in high-risk patients (C) according to the administration of UDCA prophylaxis.

The relapse incidence did not differ significantly between the arms. The cumulative incidence at 10 years was 36% versus 37% in the groups given and not given UDCA, in the low-risk group 27% versus 26%, and in the high-risk group 54% versus 50%, respectively. There were 4 secondary cancers in the group given UDCA and 7 in the control group.

The causes of death after 1 year post-transplantation in the study groups given and not given UDCA were the following (number of patients): GVHD 4 versus 4, infection 5 versus 6, relapse 17 versus 10, EBV lymphoproliferation 0 versus 1, secondary malignancy 2 versus 0, and other (respiratory insufficiency, pancreatitis, myocardial infarction, heart failure, suicide) 4 versus 3.

A landmark analysis, carried out in patients surviving at 1 year post-transplantation, did not reveal any significant differences between the study groups in relapse rate, NRM, disease-free survival, or overall survival (details not shown).

DISCUSSION

The improved survival of patients given UDCA prophylaxis was due to reduced NRM in the early phase, during the first year, and this effect was maintained throughout the 10-year follow-up period. The reduction of NRM was mainly based on reduced incidence of liver toxicity and severe acute GVHD. In a landmark analysis among patients alive at 1 year, no significant differences in the outcome parameters were observed between the study groups during the long-term follow-up. UDCA did not cause any short- or long-term adverse effects.

Limitations of this report are the retrospective nature of the study as well as the open-label design of the trial. However, the main endpoints, survival and NRM, are robust and not likely to have been significantly affected by the potential weaknesses of the study structure. Multiple testing of clinical outcomes has been taken into account in the statistical analysis.

The reported clinical effects of UDCA prophylaxis have been somewhat inconsistent [15-20]. This may be due to differences in patient population, conditioning, other components of treatment, outcome in the comparator arm, and the size of the studies. In a systematic review and pooled analysis of controlled clinical trials of the prophylactic use of UDCA, Tay et al. [20] found a significantly reduced proportion of patients with hepatic veno-occlusive disease as well as significantly reduced transplant-related mortality. There was

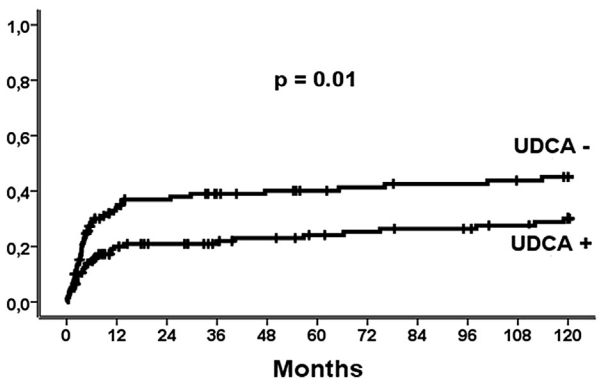

Figure 2. NRM according to the administration of UDCA prophylaxis.

a nonsignificant trend toward a lower incidence of acute GVHD. No significant difference was seen in survival. In the present study, we did not observe any effect of UDCA on the incidence of veno-occlusive disease, likely due to the overall low incidence in our patient material. An important reason may also be the conditioning, which was mainly based on total body irradiation; only a few patients received busulfan. In the present study, the reduced incidence of NRM by UDCA prophylaxis also resulted in improved survival. In the pooled analysis by Tay et al. [20], there was a trend toward better survival, but no significant effect was seen. The difference is possibly due to patient numbers; the present study is the largest one published on the use of UDCA prophylaxis in allogeneic transplantation.

The benefits from UDCA were seen in the low-risk group, whereas there was only a small nonsignificant trend in the high-risk group [1]. The reasons for this may not be fully obvious, but a possible explanation could be that the increased risks of the disease itself and the toxicity burden from the preceding treatments may dilute the beneficial effects of UDCA. There appears to be no reason to limit UDCA prophylaxis to low-(disease) risk patients. The benefits in an individual patient are not easily predictable, the disease-based stratification may not have been optimal for the prediction of the effects of UDCA, no negative effects were seen on any outcome parameter, practically no side effects occurred, the oral administration is easy, and the drug is inexpensive.

In the present trial, the administration of UDCA was initiated on the day preceding the first dose of conditioning. It is known, however, that in healthy adults it takes several days of UDCA therapy to achieve steady-state conditions for UDCA enrichment in the bile acid pool [21]. Therefore, it might be logical to initiate UDCA prophylaxis earlier before the transplant procedure.

In conclusion, the present results show that, in addition to short-term benefits, UDCA prophylaxis improves long-term survival and reduces NRM without causing any adverse effects.

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